Immunoediting and precision biomarkers in prostate cancer: The emerging role of liquid biopsy, immune contexture and HLA genotype (Review)

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Abstract. Prostate cancer (PCa) remains a leading cause of cancer-related mortality in men despite advances in screening and localized treatment. The clinical heterogeneity of PCa, ranging from indolent disease to aggressive, lethal phenotypes, underscores the urgent need for reliable biomarkers that improve diagnosis, prognostication and therapeutic decision-making. While prostate-specific antigen testing has reduced mortality, its limited specificity has resulted in overdiagnosis and overtreatment. The present review provides a comprehensive overview of contemporary and emerging biomarkers that support a precision-medicine approach to PCa management. The present review summarizes established and novel diagnostic, prognostic and predictive biomarkers, including serum- and urine-based assays, genomic and transcriptomic signatures and multiparametric imaging. Particular emphasis is placed on liquid biopsy technologies (circulating tumor cells, circulating tumor DNA and extracellular vesicles), which offer minimally invasive, real-time insights into tumor burden, molecular evolution and treatment resistance, although their clinical implementation remains context-dependent and is currently most established in advanced disease settings rather than routine early-stage management. The present review discusses the strengths and limitations of these platforms, highlighting disease-stage dependency, technical variability and sensitivity constraints. Beyond tumor-intrinsic markers, tissue-based immune biomarkers that capture the tumor immune microenvironment, including immune cell density, spatial organization, checkpoint expression and immune-related gene signatures, are explored. Evidence indicates that 'immune-hot' tumors characterized by $CD8^+$ T-cell infiltration and interferon-γ signaling are associated with improved outcomes, whereas immunosuppressive macrophage- or regulatory T-cell-dominant profiles predict poor prognosis. However, these associations are not uniform across studies and PCa remains largely resistant to immunotherapy, underscoring the need to improve the understanding of immune evasion mechanisms and the contextual limitations of immune biomarkers. Furthermore, the present review examines the emerging role of germline human leukocyte antigen class I genotype as a prognostic and predictive biomarker, explicitly integrating it with tissue-based immune contexture and liquid biopsy readouts by proposing immunoediting as a unifying mechanistic framework that links allele-specific antigen presentation to immune infiltration. Finally, the present review highlights the prognostic significance of preexisting tumor-antigen-specific $CD8^+$ T cells, which reflect an active antitumor immune response and predict a favorable progression-free survival and responsiveness to immunotherapeutic strategies. Collectively, the present review underscores the need for standardized, multimodal biomarker integration and prospective validation to enable personalized, immune-aware management of PCa.

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1. Introduction

Although the prostate cancer (PCa) mortality rate has approximately halved since the early 1990s, it still remains the second most common cause of cancer-related death in men (1,2). Current treatments for disease confined to the prostate are effective in a number of cases, yet outcomes for advanced, metastatic disease have not meaningfully improved (1-3). Among males initially diagnosed with localized prostate cancer, 20-50% encounter biochemical recurrence across the initial decade and 17-20% ultimately advance to metastatic illness, while 10-20% eventually develops advanced or castration-resistant prostate cancer (4,5). For this reason, reliable prognostic biomarkers are urgently needed to guide treatment decisions, maximize clinical benefit and limit unnecessary toxicity.

Serum prostate-specific antigen (PSA) is the cornerstone of PCa screening but it is hampered by limited specificity and an inability to reliably separate benign from malignant tumors. Although PSA screening has contributed to lower PCa mortality, it has also sparked concerns about overdiagnosis and resultant overtreatment, since distinguishing indolent from aggressive disease remains challenging (1,6,7). To improve diagnostic precision, composite approaches such as the prostate health index (PHI) and the 4Kscore, integrate multiple PSA-related parameters with clinical variables (for example, digital rectal examinations findings, prostate volume or PSA density and patient age), thereby enhancing risk stratification (8).

Numerous candidate markers have emerged that show promise in detecting clinically significant PCa and predicting tumor behavior. The present review summarizes the available non-invasive assays used in PCa screening and diagnosis, highlights biomarkers that aid the stratification of PCa aggressiveness and discusses novel markers with potential to improve diagnostic accuracy and patient outcomes. A focused literature search using PubMed and Scopus was performed to identify studies on biomarkers aimed at refining detection and risk stratification in PCa.

2. Methodology for reference acquisition and selection

For the acquisition and selection of the appropriate bibliographic references, the PubMed/NCBI (https://pubmed.ncbi.nlm.nih.gov) and the Scopus (https://www.scopus.com) databases were reviewed using the appropriate key words. Separately, the Google scholar engine (https://scholar.google.gr) was also investigated for the acquisition of further relevant articles. Due to the complexity of the topic and the diverse data that had to be collected, various combinations of key words were applied. However, since the present review was not meant to be a systematic review, a less rigorous methodology was followed. The key words included: 'Prostate cancer', 'prostate neoplasms', 'prostate tumors', 'PSA', 'androgen receptor', 'early detection', 'screening', 'biomarkers', 'prognostic biomarkers', 'diagnostic biomarker', 'prostate cancer diagnosis', 'predictive biomarkers', 'resistance biomarkers', 'liquid biopsy', 'circulating tumor cells', 'circulating tumor DNA', 'cell-free DNA', 'exosomes', 'small extracellular vesicles', 'cell-free RNA', 'imaging techniques', 'castration-resistant', 'tumor heterogeneity', 'tumor microenvironment', 'tumor infiltrating cells', 'immune contexture', 'immunotherapy', 'HLA', 'immunoediting', 'risk stratification' 'treatment response' and 'therapy resistance'. An example of PubMed/Scopus literature research applying the Boolean logic (AND/OR/NOT) included the following: ((prostatic neoplasms) OR (prostate cancer)) AND ((screening) OR (liquid biopsy) OR (circulating tumor cells) OR (cell-free DNA)) AND ((prognostic biomarkers) OR (diagnostic biomarkers) OR (risk stratification) OR (therapy resistance)).

To limit the literature range to highly relevant publications, the following inclusion criteria were applied: i) Articles written in English; ii) articles published in the last 25 years (between 2000-2025); and ii) articles with the aforementioned key words in the manuscript title. Based on these search criteria, a different number of relevant articles emerged for different key word combinations, which were individually checked for suitability and relevance to the topic. Subsequently, the remaining studies were carefully read and appropriately combined to synthesize the main topics of the present review.

3. Contemporary biomarkers in PCa

Current PCa screening relies on serum PSA levels, which help with early diagnosis and thus reduce mortality, but suffer from low specificity and false positive results. Newer diagnostic tests improve accuracy and reduce unnecessary biopsies by combining molecular and genetic/epigenetic information. Specifically, the PHI [total PSA (tPSA), free PSA (fPSA) and precursor p2PSA] and the 4Kscore [tPSA, fPSA, human kallikrein 2 (hK2) and PSA] improve the prediction of clinically significant cancer types (9-12); SelectMDx (distal-less Homeobox 1 and Homeobox C6 mRNA in urine), ConfirmMDx (methylation levels of the adenomatous polyposis coli, glutathione S-transferase Pi 1 and ras association domain family member 1 genes), PCa antigen 3 (PCA3; urinary mRNA), MiPS (transmembrane serine protease 2 fused to ETS-related gene, PSA and PCA3) and ExoDx (urine exosome mRNA) further refine risk assessment and lower overdiagnosis as well as overtreatment (13-20). Multiparametric magnetic resonance imaging (mpMRI) also shows high sensitivity, especially for larger/higher-grade tumors (21). Overall, these tools integrate molecular, genetic and imaging data to improve the detection of clinically significant PCa cases and reduce unnecessary, likely invasive, procedures.

Prognostic assays estimate tumor aggressiveness and recurrence risk to further guide treatment intensity. Examples include OncotypeDX Genomic Prostate Score (17-gene score), Prolaris (cell-cycle progression gene panel/score), ProMark (12-protein proteomic panel), DNA ploidy measures and Decipher (22-gene signature predicting systemic progression) (22-29). These tests help identify patients suitable for active surveillance vs. those who need definitive or adjuvant therapy (for instance, Decipher may guide adjuvant radiation decisions) and can reduce overtreatment while personalizing patient care.

Predictive biomarkers aim to guide therapy and monitor response-to-treatment in advanced disease. Circulating tumor cells (CTCs), cell-free DNA (cfDNA), cell-free RNA (cfRNA) and circulating tumor DNA (ctDNA) provide non-invasive,

real-time insights into metastatic PCa. cfDNA reveals androgen receptor (AR) gene alterations and has been linked to response to AR-targeted therapies (such as abiraterone and enzalutamide) (30-32). However, technical and detection limitations (including sensitivity, platform variability and interpretation) remain barriers before its routine clinical adoption. Despite challenges, these markers hold promise for precision treatment selection and monitoring in metastatic settings.

Diagnostic, prognostic and predictive biomarkers, together with imaging, are shifting PCa care from one-size-fits-all PSA screening toward more precise, personalized decision-making. Although some assays still face technical and interpretive limitations, they assist improved detection of clinically significant cancer types and treatment selection; however, for several emerging biomarkers, including liquid biopsy approaches, robust prospective validation and demonstration of clinical utility in guideline-directed care remain ongoing requirements before widespread adoption.

4. Liquid biopsy-based biomarkers

Instead of invasive tissue sampling, liquid biopsy provides a minimally invasive window into a tumor's biology by detecting cancer-derived material in the blood in the form of CTCs, cfDNA and its constituent, ctDNA, and extracellular vesicles (EVs) such as exosomes. This approach can be used for population screening and early cancer detection, for predicting clinical outcomes, for guiding treatment selection and for tracking therapy response in real time. Recent technological progress in these blood-based assays has revolutionized PCa diagnosis and accelerated biomarker discovery. Despite rapid technological advances, most liquid biopsy approaches have not yet been incorporated into routine diagnostic pathways in major clinical guidelines and are primarily used in advanced disease, clinical trials or selected decision-making scenarios.

Liquid biopsy platforms (including detecting CTCs, ctDNA and EVs) provide valuable biological insights into prostate tumor evolution and have demonstrated prognostic and, in selected contexts, predictive potential, particularly in advanced disease (33-37). However, their routine clinical implementation remains limited. Current guidelines, including the National Comprehensive Cancer Network Prostate Cancer Clinical Practice Guidelines (38) and European Society for Medical Oncology & Precision Medicine Recommendations (39), support their use mainly in metastatic settings or when tissue is unavailable, while applications in early detection, risk stratification and minimal residual disease remain investigational. Key barriers include disease stage-dependent sensitivity, assay variability, lack of standardization, biological confounders and the need for prospective evidence demonstrating clinical utility and cost-effectiveness.

CTCs. CTCs are the most established circulating cellular biomarker in PCa. Their enumeration using validated platforms (such as CellSearch) shows strong prognostic value in metastatic disease, with higher counts correlating with a shorter progression-free survival (PFS) and overall survival (OS) times (40,41). Molecular characterization of CTCs further provides predictive and mechanistic insights beyond counts. In metastatic castration-resistant PCa (mCRPC), baseline CTC counts and levels after one therapy cycle are associated with shorter PFS and OS times (33). Similarly, in newly diagnosed metastatic hormone-sensitive PCa, elevated baseline CTC counts correlate with poorer outcomes and treatment responses, confirming prognostic value across disease stages (42). Molecular profiling adds further value. For example, prostate-specific membrane antigen (PSMA) expression on CTCs is independently associated with a poorer OS and PFS (43). CTC assays can also detect actionable alterations such as AR splice variants (including AR-V7) and AR amplification linked to resistance to AR-targeted therapies, helping guide treatment in mCRPC (44-47). AR-V7 transcripts are detectable not only in CTCs but also in exosomal and plasma cfRNA (36,44,48), suggesting that combined CTC and cfRNA analysis from a single sample may provide a more comprehensive assessment.

Beyond prognosis, CTCs help forecast therapeutic response; their dynamic nature enables near-real-time monitoring of tumor evolution and treatment effects (34,35). Identifying distinct CTC subpopulations can reveal resistance mechanisms and guide therapy selection (49,50). Integrating CTC profiling with other biomarkers, such as cfDNA and microRNAs (miRNAs), improves diagnostic and prognostic accuracy (51). Despite advances in CTC technologies, challenges remain, particularly in detection sensitivity. Even CellSearch, the only FDA-approved platform, has limited sensitivity, missing approximately half of advanced cancer cases and showing similar detection rates in non-cancer and early-stage disease (52-54).

A key limitation is reliance on EpCAM for CTC capture. Tumor cells often undergo epithelial-to-mesenchymal transition, during which EpCAM expression can be reduced or lost. As a result, EpCAM-low or -negative CTCs are not efficiently captured, leading to underestimation of total CTC burden. These populations are clinically relevant, often enriched in aggressive, stem-like or therapy-resistant phenotypes [reviewed in (55-57)]. Overlooking them may bias both quantitative counts and qualitative characterization. Moreover, EpCAM expression is heterogeneous across patients and over time, further limiting EpCAM-based assays (58-62).

Altogether, CTCs are powerful, minimally invasive biomarkers in PCa, providing robust prognostic information and clinically actionable molecular insights, particularly regarding treatment resistance. However, current EpCAM-based detection methods lack sensitivity and fail to capture key mesenchymal and therapy-resistant CTC subpopulations, limiting their reliability. Integrating improved CTC technologies with complementary circulating biomarkers such as cfDNA and cfRNA is essential to achieve a comprehensive and accurate real-time assessment of disease evolution and therapeutic response. Notably, while CTC enumeration (such as via CellSearch) is the most clinically validated liquid biopsy modality and is acknowledged in guidelines as a prognostic biomarker in metastatic disease, its role remains largely limited to risk stratification and treatment monitoring rather than directing standard-of-care therapeutic decisions.

ctDNA. In PCa, ctDNA carries tumor-specific genomic and epigenomic alterations. Highly sensitive liquid-biopsy assays, such as digital droplet PCR (ddPCR) and deep next-generation

sequencing, can detect ctDNA at very low levels. For example, multiplex ddPCR panels identify AR-V7 splice variants, point mutations and AR amplifications at low copy numbers, while cfMeDIP-seq distinguishes localized from metastatic disease with high accuracy (63). Fragmentation patterns (such as long/short fragment ratios) are also being explored as ctDNA signatures (63). In metastatic disease, ctDNA levels reflect tumor burden, as plasma ctDNA fraction correlates with metastatic volume and is highest in patients with visceral metastases (64). High ctDNA burden has clear prognostic value; in large mCRPC cohorts, elevated baseline ctDNA predicts shorter PFS and OS times. For instance, in the phase III Alliance A031201 trial, ctDNA-positive patients had a significantly poorer median OS time than ctDNA-negative patients (29.0 vs. 47.4 months) (36). In localized disease, ctDNA detection after curative therapy indicates minimal residual disease (MRD) and predicts biochemical relapse (64).

Beyond prognosis, ctDNA profiling guides therapy by identifying actionable alterations, such as DNA repair gene defects (including BRCA2, ATM and CDK12) linked to poly(ADP-ribose) polymerase (PARP) inhibitor sensitivity, and AR alterations associated with resistance to AR-targeted therapies (63-65). Supporting this, the SCRUM-Japan cohort study showed higher frequencies of AR alterations and homologous recombination repair defects in mCRPC, with AR abnormalities linked to shorter time-to-treatment failure (65).

A key limitation of ctDNA is its dependence on disease burden. In mCRPC, ctDNA levels are often sufficient to detect genomic alterations and track clonal evolution during therapy, enabling prediction of response or progression (36,66-68). However, in localized disease or early MRD, ctDNA levels are often near detection limits, requiring ultra-deep sequencing, error-suppression methods (such as UMIs) and advanced bioinformatics (68). Patel *et al* (69) reported that ctDNA is often undetectable in localized PCa but increases with disease burden. Conversely, in metastatic PCa, combined targeted and low-pass whole-genome sequencing can detect ctDNA in most baseline samples, with detection rates correlating with therapy line and CTC counts (70).

Overall, ctDNA represents a promising, non-invasive biomarker that reflects tumor burden and molecular status in advanced PCa; however, its clinical utility is strongly disease-burden dependent. That is, ctDNA is routinely informative and actionable in mCRPC but often falls below the detection limit in localized disease or early MRD, where tumor-informed ultra-deep sequencing with UMIs, orthogonal validation and tailored bioinformatics are required. Remaining barriers, pre-analytic and analytic variability, tissue-plasma discordance and confounding by clonal hematopoiesis, require standardization and prospective outcome studies. Altogether, ctDNA is currently a clinically useful adjunct biomarker in selected advanced PCa settings, particularly for molecular profiling and treatment selection when tissue is unavailable, whereas its broader application in early-stage disease and MRD detection remains investigational pending further technical improvements and prospective clinical validation.

EVs. Exosomes are small EVs (35-125 nm) enclosed by a lipid bilayer that protects cargo such as miRNAs, proteins, lipids and viral particles; these can modulate signaling pathways in recipient cells and influence cancer development (71). Urinary exosomal proteins, especially in multi-marker panels, have shown high sensitivity and specificity in PCa, distinguishing patients from healthy controls. Chu *et al* (72) analyzed eight sex-steroid hormones in urinary exosomes from 286 participants and found that seven (DHEA, DHEAS, androstenedione, testosterone, progesterone, DHT and estrone) differed between PCa and controls and across Gleason groups. This supports urinary-exosome steroidomics as a tool for detection and stratification, although clinical translation requires further validation and standardization. Hamed *et al* (37) reviewed EV-derived metabolomic biomarkers, highlighting their diagnostic and prognostic potential as reflections of tumor metabolism. While promising metabolic signatures were identified, the field remains early and methodologically heterogeneous. EV-based metabolomics could complement other liquid biopsy approaches (such as examining ctDNA, proteins and CTCs) but requires standardization and larger studies.

In a multi-phase study of 149 patients with PCa, several urinary exosomal miRNAs (miR-21, miR-16, miR-142-3p, miR-451 and miR-636) were associated with metastatic disease. A combined score (miR-21, miR-451, miR-636 plus PSA) achieved high discrimination (AUC=0.925) and stratified recurrence-free survival (73). Elevated plasma exosomal miR-1290 and miR-375 were linked to a poorer OS in CRPC, improving prognostic models (74). Proteomic studies show that small EVs carry proteins involved in invasion, metastasis, resistance and immune modulation, identifying candidate biomarkers for diagnosis and risk stratification [reviewed in (75)]. These proteins may also reflect tumor microenvironment (TME) interactions and have translational potential as therapeutic targets, drug-delivery vehicles or vaccine components. Table I provides a concise overview of key circulating biomarkers, including CTCs, ctDNA and EVs and highlights their roles in prognosis, treatment selection and real-time monitoring of disease evolution. Table I also summarizes major molecular features such as genomic alterations, miRNAs and protein cargo. Overall, it emphasizes the clinical utility and current limitations of liquid biopsy approaches in PCa.

In summary, EVs, including urinary and plasma exosomes, carry diverse cargo (such as steroids, miRNAs, metabolites and proteins) reflecting prostate tumor biology and have yielded promising multi-marker signatures for diagnosis, stratification and prognosis. Although early studies show strong performance, evidence remains preliminary. Key priorities include standardizing workflows, validating biomarkers in large prospective cohorts and demonstrating clinical utility. If achieved, EV-based assays could complement ctDNA, CTCs and protein biomarkers and enable new therapeutic strategies. However, despite encouraging early data, EV-based biomarkers are not currently recommended in clinical guidelines for routine PCa management and their application remains largely investigational due to methodological heterogeneity and lack of standardized workflows.

Crucially, liquid biopsy analytes provide a dynamic, systemic complement to tissue-based immune profiling by capturing tumor evolution under immune pressure. Features

Table I. Liquid biopsy-based biomarkers in PCa: Clinical utility and limitations.

Biomarker	Source	Main clinical use	Key advantages	Key limitations	Current clinical role	(Refs.)
CTCs	Whole tumor cells in peripheral blood	Prognosis; treatment monitoring; detection of resistance features (such as AR-V7).	Longest clinical track record among liquid biopsies; allows both enumeration and phenotypic characterization.	Limited sensitivity, particularly outside metastatic disease; EpCAM-based capture may miss biologically relevant subpopulations; inter-platform variability.	Used as a prognostic tool in metastatic PCa but not routinely used to guide treatment decisions.	(33-35,42-47, 49-54)
ctDNA	Tumor-derived DNA fragments in plasma	Genomic profiling; identification of actionable alterations (such as HRR genes); monitoring disease dynamics.	Reflects overall tumor burden; captures tumor heterogeneity; enables serial, minimally invasive sampling.	Strongly dependent on disease burden; low detectability in localized disease; confounding by clonal hematopoiesis; lack of assay standardization.	Increasingly used in metastatic settings, especially when tissue is unavailable; broader use remains under evaluation.	(36,63-70)
cfRNA	Circulating RNA (including splice variants)	Detection of AR-V7 and other resistance-associated transcripts.	Provides transcriptional information not captured by DNA-based assays; can complement CTC analysis.	RNA instability; pre-analytical variability; limited reproducibility across studies.	Exploratory; not part of routine clinical workflows.	(36,44,48)
Extracellular vesicles/exosomes	Vesicles carrying miRNA, proteins, metabolites.	Diagnosis; risk stratification; biomarker discovery.	Rich molecular cargo; may reflect both tumor cells and tumor microenvironment interactions.	Significant methodological heterogeneity; lack of standardized isolation/analysis; limited prospective validation.	Primarily research-based at present.	(37,71-75)
Integrated liquid biopsy approaches	Combined analysis (CTCs, ctDNA and cfRNA).	Comprehensive tumor profiling; tracking tumor evolution.	Captures multiple dimensions of tumor biology (genomic + phenotypic).	Increased complexity; cost; lack of harmonized analytical frameworks.	Promising concept but not yet implemented in routine care.	(34,37,51, 71-75)

PCa, prostate cancer; AR-V7, androgen receptor splice variant 7; EpCAM, epithelial cell adhesion molecule; HRR, homologous recombination repair; miRNA, microRNA; CTCs, circulating tumor cells; ctDNA, circulating tumor DNA; cfRNA, cell-free RNA.

such as ctDNA-derived mutational landscapes, allele-specific copy-number alterations [including human leukocyte antigen (HLA) loss] and CTC phenotypic plasticity can reflect immunoediting processes in real time. Therefore, integrating liquid biopsy data with tissue immune contexture and germline HLA information enables a longitudinal view of tumor-immune interactions that cannot be achieved by any single modality alone.

5. Tissue-based immune biomarkers

Tissue-based immune biomarkers capture the local tumor-immune ecosystem shaping prognosis and treatment sensitivity. Rather than single markers, they reflect multi-dimensional features, including infiltrating leukocyte types [$CD8^+$ cytotoxic T cells, $CD4^+$ helper and $FOXP3^+$ T-regulatory cells, B cells, natural killer (NK) cells and tumor-associated

macrophages (TAMs)] and their density and spatial distribution (76,77). Spatial organization, whether infiltrating tumor nests, confined to margins, clustered in tertiary lymphoid structures or excluded, has independent prognostic value. The Immunoscore in colorectal cancer showed that CD3/CD8 density and localization strongly predict relapse and survival (78-80), with similar findings in melanoma, lung and liver cancer (79-82).

Beyond density, immune checkpoint expression [including programmed cell death-protein 1 (PD-1), programmed death-ligand 1 (PD-L1), CTLA-4, LAG-3, TIM-3 and TIGIT] across tumor and immune cells reveals immunosuppressive mechanisms (83,84). Transcriptomic profiles reflecting IFN-γ signaling, cytotoxicity, antigen presentation or myeloid suppression further classify tumors as 'hot' or 'cold' (85,86), often outperforming single-marker assays in predicting immunotherapy response (87,88). Spatial context is also critical; $CD8^+$ cells in direct tumor contact mediate cytotoxicity, whereas stromal-restricted cells are less effective. Emerging spatial-omics technologies (such as multiplex immunohistochemistry, imaging mass cytometry and spatial transcriptomics) enable high-dimensional mapping of these features and have identified conserved immune archetypes linked to treatment response (89-94).

Tissue immune signals correlate with prognosis and therapy response across cancer types. High $CD8^+$ density and IFN-γ signatures are associated with improved survival and response to immune checkpoint inhibitors (86). In PCa, an 'immune-activated' phenotype similarly predicts favorable outcomes. High tumor-infiltrating $CD8^+$ T-cell density is linked to improved survival and is an independent prognostic factor (95). In high-risk disease, increased $CD8^+$ cells, especially with low M2 macrophages, correlate with markedly improved PFS (~91% at 5 years) (96). Transcriptomic analyses further support this; a Tumor Immune Contexture Score based on immune signatures predicts longer recurrence-free survival and improves prognostication (97). Additionally, a subset of aggressive localized PCa (~25%) shows PD-L1 expression with dense $CD8^+$ infiltrates, resembling immunogenic, immune checkpoint inhibitor (ICI)-responsive tumors (98). In mCRPC, patients with high $CD8^+$ infiltration and strong IFN-γ signatures have prolonged survival after CTLA-4 therapy, whereas those lacking these features had poorer outcomes (~10 months survival) (99). Notably, not all studies confirm a uniformly beneficial role for $CD8^+$ T-cell infiltration in PCa. In some cohorts, increased immune infiltration has been associated with higher-grade disease or reflects a reactive, yet ineffective, immune response in the context of strong immunosuppressive signaling (100,101). These discrepancies underscore that immune cell presence alone is insufficient, and functional state, spatial distribution and suppressive context critically determine clinical impact.

IFN-related gene signatures may have complex prognostic implications. Chronic activation of a type-I/III IFN-stimulated gene program often coincides with aggressive disease. For example, the 'IFN-related DNA Damage Resistance Signature' (IRDS), a 49-gene IFN-stimulated profile, was found to be elevated in prostate tumors of African-American men and was linked to poorer outcomes. In The Cancer Genome Atlas (TCGA) data, tumors with high IRDS had a significantly reduced disease-free survival (102). Consistently, an IFNL4 germline variant (ΔG allele) that drives IFN-λ4 production was correlated with higher IRDS expression and a poorer OS (102). However, in contrast to primary tumors, metastatic prostate lesions (especially bone metastases) often show loss of tumor-intrinsic type-I IFN signaling (103), reflecting an immune-cold, treatment-resistant state. Conversely, a strong IFN-γ signature is a hallmark of active antitumor immunity; in the aforementioned anti-CTLA-4 trial, the 'favorable' subgroup had an IFN-γ response signature, which combined with the high $CD8^+$ T-cell infiltration, notably improved their survival (99). Furthermore, IFN signaling exhibits context-dependent effects, as chronic or dysregulated activation may promote immune exhaustion, tumor adaptation and resistance to therapy, thereby complicating its interpretation as a uniformly favorable biomarker.

Enrichment of tumors with suppressive T-cell subsets correlates with a poor prognosis. Tumors destined to relapse often show high levels of $FOXP3^+$ regulatory T cells (Tregs) along with M2 macrophages (104). In general, most analyses report that abundant $CD4^+$ and $CD8^+$ T-cell gene signatures are linked to a longer recurrence-free survival and OS, whereas high Treg signatures mark more aggressive disease (105). Conversely, microenvironments dominated by myeloid-derived suppressor cells (MDSCs) or TAMs, often leading to T-cell exclusion, predict poor therapeutic outcomes (85,106). However, in PCa, immune signals are typically weaker and more heterogeneous. Subsets of patients with microsatellite instability, high tumor mutational burden or DNA-repair defects exhibit heightened sensitivity to PD-1 blockade (107-110). Elevated PD-L1 expression and the presence of $PD\text{-}1^+$ lymphocytes are associated with aggressive disease, but PD-L1 alone has not emerged as a reliable predictive biomarker across most PCa cohorts (98,111). Gene signatures reflecting macrophage infiltration generally predict poorer outcomes. Several reports have revealed that prostate tumors with a dominant M2-macrophage signature have faster progression. For instance, a TCGA-based model found that M2-macrophage genes were significantly enriched in the high-recurrence-risk group (112). Another immune-subtyping study classified PCa into high-cytolytic, high-M2-TAM and high-Tregs subclasses; the high-M2-TAM subtype showed the worst clinicopathological features and the lowest recurrence-free survival (113). In line with this, increased infiltration of both M1 and M2 macrophages has been associated with a shorter survival time (104,105,113). Thus, an 'immune-suppressive' macrophage profile, especially a M2-biased signature, consistently marks patients with a poorer prognosis (such as earlier relapse and death) (96,113). However, the classification of immune subsets into strictly 'favorable' or 'unfavorable' categories may oversimplify a highly dynamic system as macrophage polarization and T-cell functional states exist along continua and can shift during disease progression or in response to therapy.

Altogether, 'immune-hot' signatures (such as high $CD8^+$ T-cell/cytolytic and IFN-γ response gene expression) characterize prostate tumors with improved outcomes, whereas 'immune-suppressive' signatures (such as high M2-macrophage, high Tregs and chronic IFN-I/III stimulated programs) are linked to earlier recurrence, metastasis and poorer survival. These findings indicate that the composition

of the tumor immune microenvironment, as captured by cell- and gene-expression signatures, has notable prognostic value in both localized and advanced PCa. Table II summarizes the key immune cell populations, spatial organization, checkpoints and gene signatures within the TME and highlights their prognostic and predictive value, emphasizing how tissue immune context can inform prognosis and immunotherapy response in PCa.

Notably, these tissue-derived immune phenotypes are not isolated observations but reflect the downstream consequences of antigen presentation constraints imposed by the host germline HLA repertoire and tumor-intrinsic antigen-processing capacity. Thus, spatial immune patterns such as 'hot' vs. 'cold' tumors can be interpreted as phenotypic readouts of ongoing immunoediting, linking tissue-based immune biomarkers mechanistically to HLA genotype and to the evolutionary trajectories captured by circulating biomarkers. Nevertheless, the prognostic and predictive value of these immune features in PCa is less consistent than in highly immunogenic tumors such as melanoma or lung cancer (114), reflecting the generally 'immune-cold' nature of prostate tumors and highlighting important biological and methodological variability across studies.

Caveats with immune-based signatures. Despite the biological promise of immune biomarkers, several clinical and technical challenges limit their routine implementation. Sampling bias and spatial heterogeneity mean that immune infiltration can vary markedly within and between tumor sites, leading to potential misclassification when relying on small bioptic material (112,115). Pre-analytical factors such as fixation time, ischemia and storage conditions can degrade epitopes and RNA, altering staining quality (116,117). Moreover, assay variability, including differences in antibody clones used, platforms and scoring systems, produces inconsistent results across laboratories, as underscored by harmonization studies performed for PD-L1 (118-121). Additionally, checkpoint molecule expression is dynamic and can shift in response to prior treatments such as androgen deprivation, radiotherapy or chemotherapy, making the timing of tissue sampling critical (122,123).

In PCa specifically, PD-1 and PD-L1 are more frequently detected in high-grade or locally advanced tumors, yet their prognostic associations vary widely due to assay and cohort differences (124,125). While checkpoint blockade has revolutionized therapy in melanoma and lung cancer, trials in PCa have yielded mixed outcomes. For example, the CA184-043 phase III trial of ipilimumab following radiotherapy did not achieve its primary survival endpoint but revealed potential benefit in defined subgroups (126,127). Combination regimens targeting PD-1 and CTLA-4 or integrating PARP inhibitors in DNA-repair-deficient tumors, have shown occasional durable responses, emphasizing the need for more refined predictive biomarkers (128,129). Meanwhile, prostate-specific targets are being explored for immune-based therapies, including chimeric antigen receptor (CAR)-T cells (130-134), bispecific T-cell engagers (135-138) and radioligand approaches (139-141), with early studies showing proof-of-concept efficacy.

To translate tissue-based immune biomarkers into clinical decision-making tools, several elements must converge, such as standardized pre-analytical tissue handling, validated staining protocols, digital scoring algorithms using automated image analysis and cross-platform harmonization of antibody clones and RNA panels. Furthermore, rigorous prospective validation within randomized trials or preplanned cohorts is essential. The Immunoscore framework in colorectal cancer provides a successful precedent for analytical validation and clinical translation; however, PCa will likely require tumor-specific adaptations that integrate spatial and transcriptomic data alongside multimodal biomarker readouts (142,143).

Beyond technical limitations, a major biological challenge is that immune biomarkers, such as PD-1/PD-L1, in PCa often fail to translate into effective therapeutic responses (144,145). Despite evidence of immune infiltration or checkpoint expression, most patients do not derive durable benefit from ICIs, highlighting a disconnect between biomarker presence and functional antitumor immunity. This discrepancy emphasizes the need to better define which immune features are merely correlative vs. those that are mechanistically linked to therapeutic response.

6. HLA-A alleles as prognostic and predictive biomarkers in PCa: The role of immunoediting

The HLA class I genotype is increasingly recognized as a factor associated with cancer outcome and response to immune-based therapies, although causal relationships remain to be definitively established. Notably, HLA genotype represents an upstream, host-intrinsic layer of biomarker information that functionally connects with both tissue-based immune contexture and circulating tumor-derived signals. Multiple studies report that the presence of specific HLA alleles (and HLA-I zygosity/supertypes) correlates with survival differences across tumor types, and the HLA genotype can shape responses to immune-checkpoint blockade (146-149). Recent work from our group showed that HLA-A24 is associated with a favorable outcome, whereas HLA-A2 is associated with a poorer prognosis in early-stage and metastatic PCa (150,151). These allele-level effects can be interpreted in the context of tissue immune infiltration patterns and liquid biopsy features, suggesting that the HLA genotype contributes to shaping both the tumor immune microenvironment and the circulating tumor evolutionary landscape. The prognostic signals at the allele-level have been observed in independent cohorts and suggest that the HLA-A genotype may contribute to patient risk stratification beyond standard clinicopathologic variables, although these observations require independent validation and mechanistic confirmation (150). Reports across other malignancies produce a mixed pattern; HLA-A2 has been linked to poorer prognosis in ovarian and lung cancer (146,148), while in breast cancer and melanoma HLA-A2 appears neutral (147,152). Conversely, HLA-A24 has been reported among unfavorable prognosticators for melanoma and lung cancer (147,153), in contrast to its favorable signal in PCa cohorts (150,151,154). These tumor-dependent discrepancies indicate that the mechanisms by which particular HLA-A genotypes influence clinical outcome differ among cancer types and are shaped by tumor biology.

Table II. Tissue-based immune biomarkers in prostate cancer: interpretation and limitations.

Biomarker category	Components assessed	Reported associations	Strengths	Limitations	Practical interpretation	(Refs.)
Immune cell infiltration	$CD8^+$ T cells, $CD4^+$ T cells, $FOXP3^+$ Tregs, macrophages and NK cells	$CD8^+$ T cells are often linked to improved outcomes, whereas Tregs and M2 macrophages are generally associated with poorer prognosis.	Directly reflects immune presence within the tumor	Associations are not consistent across cohorts; does not capture functional state of immune cells.	Requires contextual interpretation (density alone is insufficient).	(76,77,96,105, 106,112)
Spatial immune organization	Localization (intratumoral vs. stromal vs. invasive margin); tertiary lymphoid structures.	'Immune-inflamed' patterns tend to correlate with improved outcomes; excluded/desert phenotypes with resistance.	Adds spatial context, which is critical for interpreting. immune function	Technically demanding; limited standardization across studies.	Increasingly recognized as a key determinant of immune effectiveness.	(78-82,96,97)
Immune checkpoint expression	PD-1, PD-L1, CTLA-4, LAG-3, TIM-3 and TIGIT.	Often associated with aggressive disease; predictive value for immunotherapy remains limited in PCa.	Clinically actionable targets; widely studied .	Dynamic and treatment-dependent; assay variability; poor standalone predictive. value	Insufficient as single biomarkers in PCa.	(83,84,98,111)
Immune gene expression signatures	IFN-γ-related genes, cytotoxicity markers, T-cell-inflamed profiles and IRDS.	IFN-γ signatures linked to favorable outcomes; chronic IFN signaling may indicate resistance.	Captures functional immune state beyond cell counts.	Interpretation can be context-dependent; variability between datasets.	More informative than single markers but not yet standardized.	(85-94,99, 102,103)
Immunosuppressive microenvironment	Tregs, MDSCs and M2 macrophages.	Generally associated with poor prognosis and immune evasion.	Reflects mechanisms of resistance.	Overlapping phenotypes; difficult to quantify reproducibly.	Important for understanding lack of immunotherapy response.	(104-106,112)
Immunogenic tumor subsets	MSI-high, high TMB and DDR-deficient tumors	Enriched for response to immune checkpoint inhibitors.	Clinically actionable in selected cases	Rare in prostate cancer; limited generalizability.	Relevant for a small subset of patients.	(107-110,113)

NK, natural killer; Tregs, regulatory T cells; PCa, prostate cancer, IFN, interferon, MDSCs, myeloid-derived suppressor cells, MSI, microsatellite instability; TMB, tumor mutational burden; DDR, DNA damage and repair; PD-1, programmed cell death protein 1; PD-L1, programmed cell death ligand 1; LAG-3, lymphocyte activation gene 3; TIM-3, T-cell immunoglobulin and mucin-domain containing 3; TIGIT, T-cell immunoreceptor with Ig and ITIM domains; IRDS, IFN-related DNA Damage Resistance Signature.

Mechanistic explanations that might drive allele-specific prognostic effects. Immunoediting, a dynamic process in which the immune system i) eliminates highly immunogenic tumor cells, ii) establishes an equilibrium that shapes tumor composition and iii) ultimately drives immune selection leading to escape from immune surveillance, provides a mechanistic framework that unifies tissue-based immune phenotypes and liquid biopsy observations. Within this framework, tissue-level features such as $CD8^+$ T-cell infiltration or immune exclusion represent spatial endpoints of immunoediting, whereas liquid biopsy signals (such as ctDNA-detected clonal evolution, CTC heterogeneity or emerging resistance alterations) reflect their temporal dynamics. This contributes to our understanding of how host and tumor alleles might contribute to allele-specific prognostic associations, rather than establishing direct causal relationships (155). While this framework is supported by biological plausibility and indirect evidence, definitive causal links between specific HLA alleles, immunoediting processes and clinical outcomes in PCa remain to be demonstrated. In principle, immunoediting is allele-dependent since the recognition of tumor-derived peptides by adaptive and innate effectors depends on germline-encoded molecules (notably classical HLA alleles, but also NK receptors and Fc receptors) and on the tumor-encoded antigen-processing and presentation machinery. Over time, allele-dependent recognition and selection reshape tumor clonality and the tumor-immune microenvironment, producing measurable differences in recurrence, survival and therapy sensitivity between patients with different germline or tumor allele patterns (155-159). The first step for mechanistic links between immunoediting, immune selection and allele-specific prognosis is somatic mutation, generating peptides (neoantigens) whose ability to be presented to T cells is determined by the HLA repertoire of each patient and by the integrity of the antigen-processing pathway (160-162). Different HLA alleles bind distinct peptide motifs with differing affinities, so an identical tumor neoepitope may be strongly and effectively presented in one patient, but stay invisible in another; when strongly presented, such neoantigens elicit the potent elimination of the expressing tumor clones and have been associated with improved tumor control and clinical outcome in several studies, although such associations are context-dependent and not uniformly observed (157,158,163). Heterozygosity or higher evolutionary divergence across HLA alleles broadens the set of peptides that can be presented and has been associated with stronger antitumor responses and, in some studies, improved outcomes, although findings are not entirely consistent across tumor types and study designs (149,159,164,165). At the same time, allele-specific effects should be interpreted cautiously, as HLA associations may be confounded by population structure, linkage disequilibrium and tumor-intrinsic features, and do not uniformly translate into effective immune-mediated tumor control in all clinical contexts.

Demonstrating allele-specific prognostic effects driven by immunoediting requires integrating germline genotyping, tumor genomic profiling, neoantigen prediction, direct peptide identification (immunopeptidomics), immune phenotyping and clinical follow-up. Paired tumor exome/RNA sequencing with germline HLA typing permits the prediction of which clonal neoantigens are presented by a patient's alleles and whether the clonal neoantigen burden correlates with outcome or response to checkpoint blockade (157,166-170). Computational tools and copy-number methods that detect allele-specific loss of heterozygosity in HLA (LOHHLA) in tumor genomes and, when combined with longitudinal sampling, reveal the acquisition of escape variants concurrent with clinical progression or relapse (158,171,172). These allele-specific escape events are increasingly detectable in ctDNA, enabling non-invasive monitoring of immunoediting in real time and linking genomic liquid biopsy readouts directly to HLA-driven immune selection pressures. Immunopeptidomics by mass spectrometry provides the most direct evidence of allele-specific presentation and can validate *in silico* predictions that otherwise overestimate the actual peptide presentation (173-175). Functional assays (T cell reactivity, peptide-HLA tetramers or engineered tumor models in HLA-transgenic mice) are critical for establishing that a given peptide-allele pair elicits cytotoxic responses and that loss of that allele abrogates killing (176,177). Notably, much of the current evidence is derived from retrospective analyses, computational neoantigen predictions or correlative immune phenotyping, and prospective studies with functional validation remain limited.

From a statistical perspective, models must adjust for key confounders (tumor mutational burden, neoantigen clonality, clonal vs. subclonal variants/mutations, tumor stage, microsatellite instability status, therapy type and patient ancestry) and should test interactions during the course of the disease to show whether the prognostic effect of a tumor feature depends on host alleles (178). Longitudinal analyses are particularly informative; considering tumor allele changes (for example, acquisition of LOHHLA or β2-microglobulin mutations) as time-dependent covariates clarifies directionality and helps discriminate cause from consequence (179). Finally, integrating orthogonal data types such as immunopeptidomics, allele-specific expression at the RNA level and T cell functional data, reduces false positives inherent to binding prediction algorithms and strengthens causal inference (157,158,180). Such integrative approaches will deepen mechanistic understanding of tumor-immune crosstalk and enable more precise prognostic biomarkers and allele-aware immunotherapeutic strategies, particularly when HLA genotype is analyzed in conjunction with tissue immune contexture and longitudinal liquid biopsy data.

*Contrasting prognostic roles of HLA-A*02:01 and HLA-A*24:02 in PCa.* The underlying etiology of the observed association between HLA-A*02:01 and a less favorable prognosis in patients with PCa may be partially explained by the heterogenic presentation of tumor peptides specifically restricted by this allele. Heterogeneous expression of tumor-derived peptides that are presented by a particular HLA allele can generate and amplify intratumoral heterogeneity variously, for instance: i) Somatic mutations encoding for neoantigens are subclonal or variably expressed across tumor regions; thus, the set of peptides available for HLA-I presentation differs between tumor subclones, producing neoantigen heterogeneity that further generates tumor heterogeneity (181-184); ii) even when a peptide-coding mutation is present, differences in the antigen-processing and presentation machinery [proteasomal cleavage, peptide

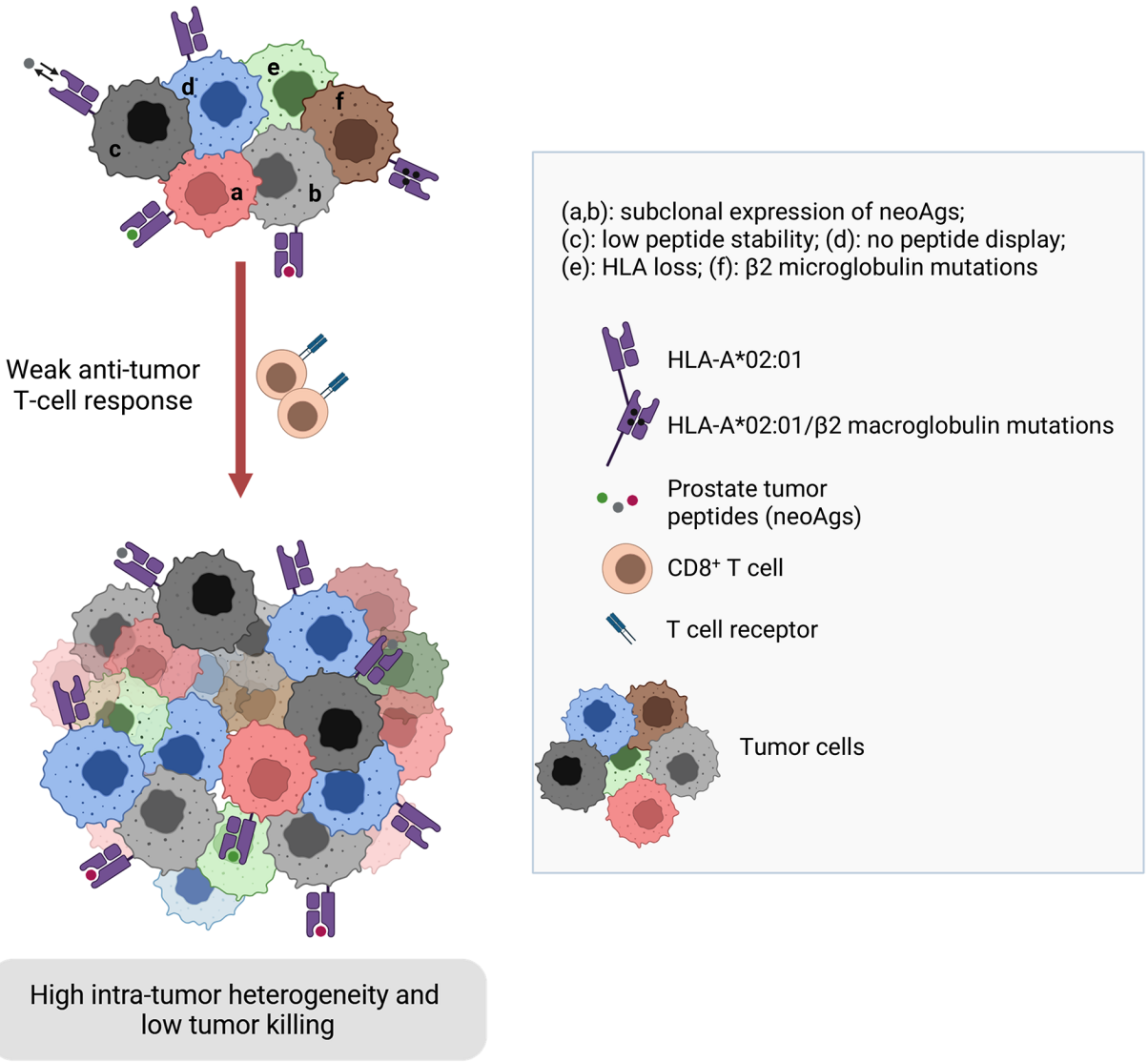

Figure 1. HLA-A*02:01 allele-restricted peptide presentation as a driver of intratumoral heterogeneity. Tumor cells expressing the same HLA allele (in this case the HLA-A*02:01 allele) can present distinct repertoires of tumor-derived peptides, generating multiple layers of intratumoral heterogeneity. (A,B) Neoantigen-encoding somatic mutations are frequently subclonal or unevenly expressed across tumor regions, resulting in spatially variable peptide availability for HLA class I presentation. (C) Even when a mutation is present, defects in antigen-processing and presentation pathways, including proteasomal cleavage, peptide trimming, transporter associated with antigen processing-mediated transport, endoplasmic reticulum loading and endoplasmic reticulum aminopeptidase editing, negatively affect the stability of peptide-HLA class I complex and its display at the cell surface. (D) Sine peptide presentation is HLA allele-specific, allele-selective loss or downregulation of HLA class I molecules (such as via loss of heterozygosity or other MHC-I alterations) produces tumor subclones that no longer present the peptide, enabling immune-mediated selection and spatial or temporal segregation of presenting and non-presenting cells. Under sustained T-cell-mediated immune pressure, tumors may acquire defects in antigen-presentation pathways, such as (E) HLA loss or (F) β2-microglobulin mutations, further increasing phenotypic heterogeneity and promoting resistance to T-cell-based therapies [Created in BioRender. Fortis, S. (2026) https://BioRender.com/mmsl3zm]. Agreement license WF29LKNSK4. HLA, human leukocyte antigen; MHC, major histocompatibility complex.

trimming, transporter associated with antigen processing (TAP)/endoplasmic reticulum peptide loading and endoplasmic reticulum aminopeptidase editing] determine the stability of the peptide:HLA class I complex and whether a given peptide is actually displayed, further contributing to HLA-specific tumor-peptide heterogeneity and diversifying presentation across cells (185-187); iii) since peptide presentation is HLA-allele specific, loss or downregulation of the particular HLA allele [for example by allele-specific LOH or other major histocompatibility complex (MHC)-I alterations] will produce subclones that no longer display the peptide, allowing immune-mediated selection of non-presenting clones and spatial/temporal segregation of peptide-positive and peptide-negative tumor cell populations (158,186,188); and iv) tumors evolving under T-cell pressure can acquire defects in antigen-presentation pathways, including HLA loss or β2-microglobulin mutations, both of which increase phenotypic heterogeneity and can drive resistance to T-cell-based therapies (189) (Fig. 1). Thus, the heterogenic expression of tumor peptides specifically restricted by HLA-A*02:01 may contribute to the observed association with less favorable clinical outcomes in patients homozygous for HLA-A*02:01 (150). Notably, such HLA-dependent heterogeneity in antigen presentation may also manifest in liquid biopsy profiles as increased clonal diversity in ctDNA and phenotypic diversity in CTC populations, thereby bridging allele-specific immune

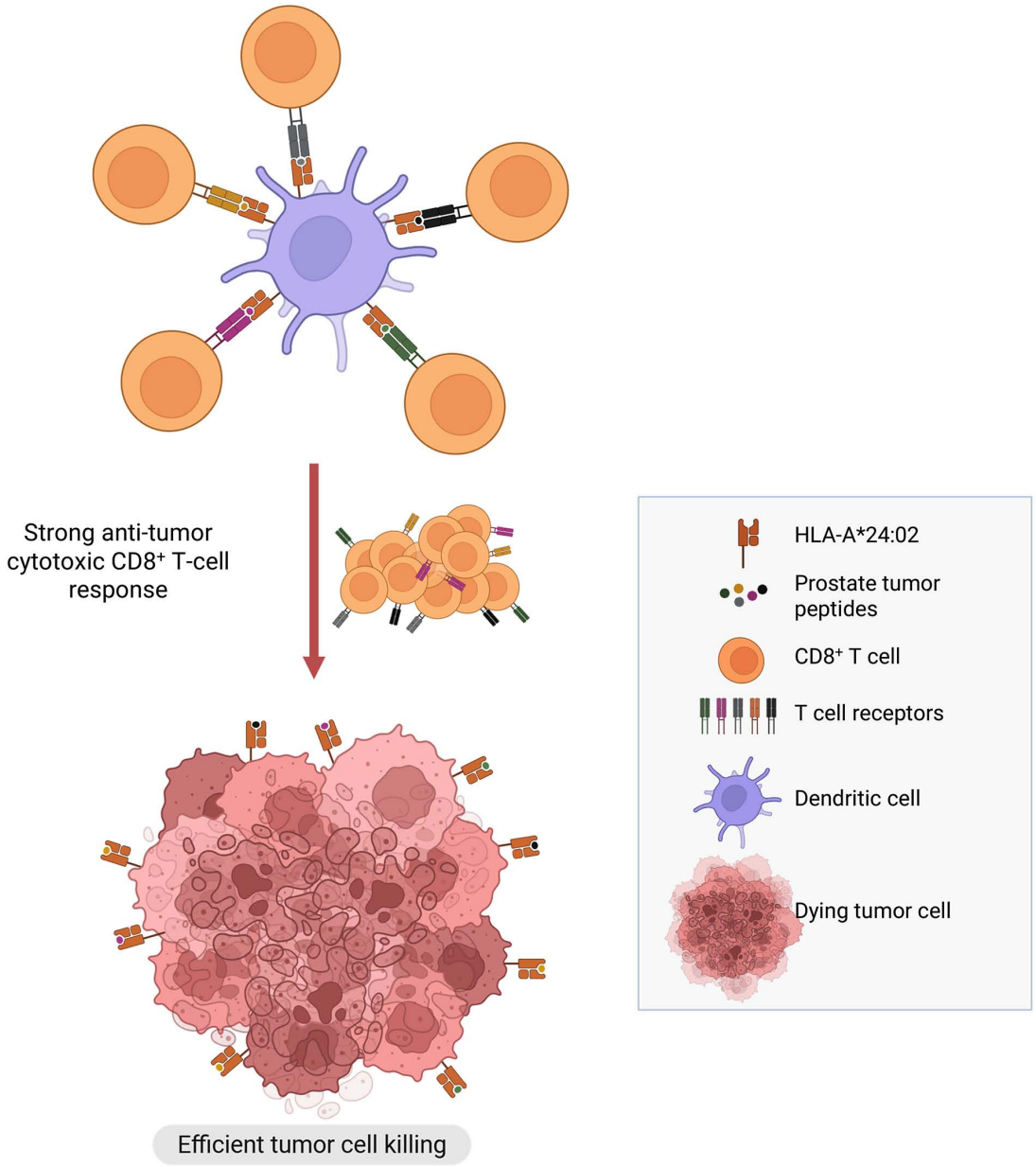

Figure 2. HLA-A*24:02-mediated presentation of immunogenic tumor peptides drives potent CD8+ T cell antitumor immunity. The schematic depicts the HLA-A*24:02 allele effectively binding and presenting tumor-derived immunogenic peptides on the cell surface, facilitating recognition by CD8+ T cells. Peptides produced via intracellular antigen processing are loaded onto HLA-A24 molecules, forming stable peptide-HLA complexes that drive strong activation, clonal expansion and effector differentiation of tumor-specific CD8+ T cells. This coordinated process of antigen presentation and CD8+ T-cell activation enhances cytotoxic activity, resulting in efficient tumor cell elimination [Created in BioRender. Fortis, S. (2026) https://BioRender.com/3v0nh8u]. Agreement license GD29LKO1LQ. HLA, human leukocyte antigen.

selection with measurable circulating tumor heterogeneity. Loss-of-expression mutations affecting HLA-A2 have been intermittently identified *in vitro* in melanoma and cervical cancer settings using conventional PCR and sequencing approaches (190-192). However, the reliable detection of somatic alterations in HLA genes by whole-exome sequencing remains technically challenging, largely due to the extreme polymorphism and complex genomic architecture of the HLA locus (193-195). These limitations likely contribute to the underrepresentation of reported HLA mutations in large-scale genomic studies of PCa.

Several plausible, non-exclusive immunological and evolutionary mechanisms have been proposed to explain why HLA-A*24:02 might be associated with improved PCa outcomes compared with HLA-A*02:01: i) HLA-A*24:02 presents immunodominant, prostate-tumor peptides that drive strong CD8+ T-cell responses. The HLA-A*24:02 allele has the capacity to efficiently bind and display tumor-derived immunogenic peptides at the cell surface, potentially enabling more effective recognition by CD8+ T cells. Peptides generated through intracellular antigen processing are loaded onto HLA-A24 molecules and presented in a stable peptide-HLA complex, promoting enhanced activation, clonal expansion, and effector differentiation of tumor-specific CD8+ T cells. Several studies have identified HLA-A24-restricted cytotoxic T-lymphocyte

(CTL) epitopes from prostate antigens [such as PSA, PSMA, HER-2/neu and telomerase reverse transcriptase (TERT)] and cancer/testis or tumor-associated antigens that are immunogenic in HLA-A*24:02 patients; therefore, these patients may be capable of mounting peptide-specific CTL responses that help control tumor growth (196-199) (Fig. 2). ii) HLA-A*24:02 may preferentially facilitate the presentation of conserved, clonally expressed tumor antigens, thereby biasing antigen display toward low-heterogeneity and functionally constrained targets. Consistent with this, tumors constrained by presentation of clonally conserved antigens might be expected to exhibit reduced clonal diversification in ctDNA analyses and a more stable immune-infiltrated phenotype at the tissue level, reinforcing the link between HLA genotype, immunoediting constraints and multimodal biomarker readouts. If HLA-A*24:02 exhibits enhanced binding affinity for peptides derived from essential oncogenic or survival-associated proteins, such as Survivin (BIRC5), BCL-2 family members or other conserved driver pathways, then these epitopes are likely to be ubiquitously expressed across all tumor cells within a lesion. As such proteins are required for tumor cell viability, proliferation or resistance to apoptosis, their loss or downregulation would impose a substantial fitness cost, limiting the tumor's ability to escape immune recognition through antigen loss or clonal deletion. Consequently, tumors arising in HLA-A*24:02-positive hosts may remain persistently visible to cytotoxic $CD8^+$ T cells, as immune editing cannot readily eliminate all relevant antigenic targets without compromising tumor fitness. In this context, immune evasion via subclonal antigen loss, HLA-restricted neoantigen depletion or immunoediting of dispensable passenger mutations would be less effective. Moreover, clonally expressed tumor peptides presented by HLA-A*24:02 are predicted to display high peptide-MHC class I binding stability and prolonged surface half-life, leading to increased peptide density on the tumor cell surface (200,201). These features promote more efficient T-cell receptor engagement, enhanced priming of antigen-specific $CD8^+$ T cells and increased immunogenicity (Fig. 3). Thus, preferential presentation of conserved, essential antigens and strong HLA-A*24:02 binding may constrain immune escape pathways and contribute to more durable antitumor immune surveillance. iii) Differential immune-editing/allele-specific loss dynamics. Tumors commonly escape T-cell pressure via allele-specific HLA loss or downregulation, but the frequency and consequences of allele-specific loss vary by tumor type and context. If HLA-A*24:02 is less commonly lost/edited in prostate tumors, or if loss of HLA-A*24:02 is more deleterious to tumor fitness, this would preserve presentation and anti-tumor immunity in HLA-A*24:02 carriers (201,202). Consequently, the favorable prognostic signal for HLA-A*24:02 in PCa is biologically plausible and can be explained by its stable expression and by presentation of a peptide repertoire that elicits stronger/stable antitumor cytotoxic $CD8^+$ T-cell responses. Moreover, the favorable and durable role of HLA-A*24:02 expression on PCa clinical outcomes might be interpreted by the increased immunogenicity of HLA-A*24:02^+ cancer cells via expression of tumor peptides throughout PCa evolution, leading to sustained activation of tumor-specific T-cell memory and subsequently to long periods of tumor control under immunosurveillance. Such an extended 'equilibrium' results in reduced tumor growth rates and increased OS (Fig. 4). Validation in larger patient cohorts is required to substantiate these observations and clarify their clinical relevance. Such efforts should be accompanied by mechanistic investigations to delineate how specific HLA class I alleles confer either adverse or favorable prognostic effects. Taken together, these proposed mechanisms are biologically plausible but remain speculative, and direct experimental evidence linking specific HLA-A alleles to distinct immunoediting processes and clinical outcomes in PCa is currently limited.

Clinical implications and caveats. The allele-specific nature of immunoediting has direct translational implications. Germline HLA genotype, HLA zygosity/divergence and detection of allele-specific tumor escape mechanisms (such as LOHHLA) and β2-microblobulin/TAP alterations) are candidate biomarkers for prognosis and for stratifying patients to immunotherapies or vaccine strategies that prioritize clonal, presented neoantigens (149,157,203,204). Monitoring the emerging allele-specific escape during or after therapy could flag imminent relapse and encourage the use of combination approaches that bypass MHC-dependent peptide recognition (for example, NK cell-based therapies) or restore antigen presentation. In this context, integrating germline HLA typing with tissue-based immune profiling and serial liquid biopsy monitoring offers a comprehensive strategy to capture both the spatial and temporal dimensions of tumor-immune interactions.

Important caveats mitigate these opportunities. Allele frequencies and tumor features co-vary with ancestry and environmental exposures; therefore, population stratification and confounding issues must be rigorously addressed. *In silico* MHC-binding predictions remain imperfect proxies for immunogenicity; predicted binding does not guarantee T cell recognition, which depends on the T-cell receptor (TCR) repertoire and the inflammatory context (168). Tumor heterogeneity and sampling bias can obscure subclonal escape events and cross-sectional studies can misattribute causality when longitudinal dynamics are overlooked. Thus, mechanistic and clinical claims require reproducibility across tumor types, direct peptide generation and presentation when possible and functional validation in experimental systems (182,188,205).

Overall, immunoediting and immune selection provide a unified, mechanistic explanation for HLA allele-specific prognostic effects in cancer. Which tumor peptides are presented (and by which germline alleles), how effectively they are recognized by adaptive and innate effectors and whether the tumor can evolve allele-specific escape collectively determine clonal architecture, immune infiltration and clinical trajectory. Clarifying these allele-dependent pathways demands multimodal cohorts that combine germline HLA and immune-receptor genotyping, tumor genomics and transcriptomics, immunopeptidomics, functional immunology and longitudinal clinical data. Such integrative studies will both deepen mechanistic understanding of the tumor-immune crosstalk and enable more precise prognostic biomarkers and allele-aware immunotherapeutic strategies.

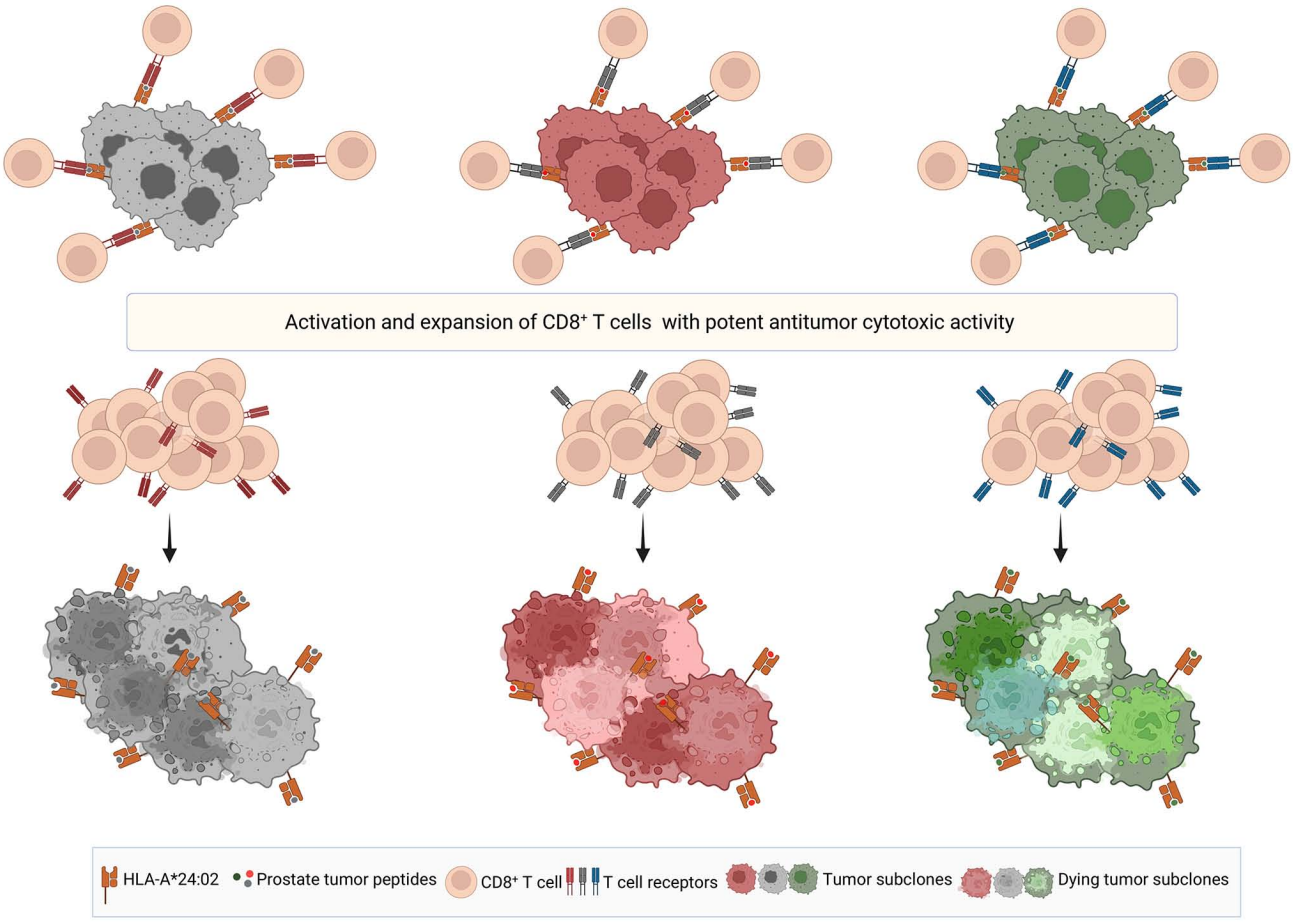

Figure 3. HLA-A*24:02 may preferentially present conserved or essential 'driver' antigens as targets with low heterogeneity. HLA-A*24:02 may preferentially facilitate the presentation of conserved, clonally expressed tumor peptides with high MHC class I binding affinity, leading to the activation and expansion of CD8+ cytotoxic T cells bearing T-cell receptors specific for these epitopes. Consequently, tumors expressing HLA-A*24:02 remain readily detectable by activated cytotoxic CD8+ T cells, which can efficiently recognize and eliminate them [Created in BioRender. Fortis, S. (2026) https://BioRender.com/lwwc6qi]. Agreement license KY29LKV4HJ. HLA, human leukocyte antigen; MHC, major histocompatibility complex.

7. Preexisting frequencies of tumor-peptide specific CD8+ T cells as prognostic biomarker in PCa

Preexisting tumor-antigen-specific CD8+ T cells represent a functional convergence point of the aforementioned biomarker layers as they depend on effective HLA-mediated antigen presentation, are reflected in tissue immune infiltration patterns and may indirectly influence circulating tumor evolution captured by liquid biopsy assays. Therapies that rely on the immune system (directly, such as ICIs or vaccines, or indirectly, such as some chemotherapy, radiotherapy, hormonal therapies and targeted agents) work far better when already an active, tumor-specific immune response is in place. Patients whose tumors are already infiltrated by antigen-specific CD8+ T cells and show a 'T-cell-inflamed' immune microenvironment possess an initial advantage as these T cells can be re-invigorated, expanded and/or helped to traffic into tumor nests so they kill cancer cells quickly. By contrast, tumors lacking preexisting immunity (preI) (so-called 'cold' or non-inflamed tumors) must first generate or recruit effective T cells, a process that is slower, less reliable and frequently blocked by immunosuppressive mechanisms (206). However, the presence of preexisting tumor-specific T cells does not invariably translate into effective tumor control as these cells may be functionally exhausted, spatially excluded or suppressed by inhibitory pathways within the TME.

The presence and location of effector T cells within the TME belong to the factors that make preI decisive. Namely, it is not just whether T cells exist, but where they are located. CD8+ T cells located at the invasive margin and within the tumor are poised to recognize and kill cancer cells; their presence predicts responses to PD-1 pathway blockade and other immunotherapies since ICIs act on these exhausted but antigen-experienced memory T cells (207). In our previous study, focusing on the predominant tumor-infiltrating lymphocyte subset, namely CD8+ T cells, patients with breast cancer and high CD8+ T-cell densities in the tumor center combined with low densities in the invasive margin exhibited the most favorable clinical outcomes. Conversely, the opposite distribution (low CD8+ densities in the tumor center combined with high CD8+ densities in the invasive margin) correlated with increased recurrence risk and poorer survival outcomes (208). Functional state and clonality represent two additional factors that regulate the strength of preI. Preexisting T cells that are clonally expanded and show effector/IFN-γ signatures are more likely to mediate tumor regression when stimulated. Conversely, T cells that are dysfunctional, excluded from the tumor parenchyma or suppressed by regulatory cells (such as

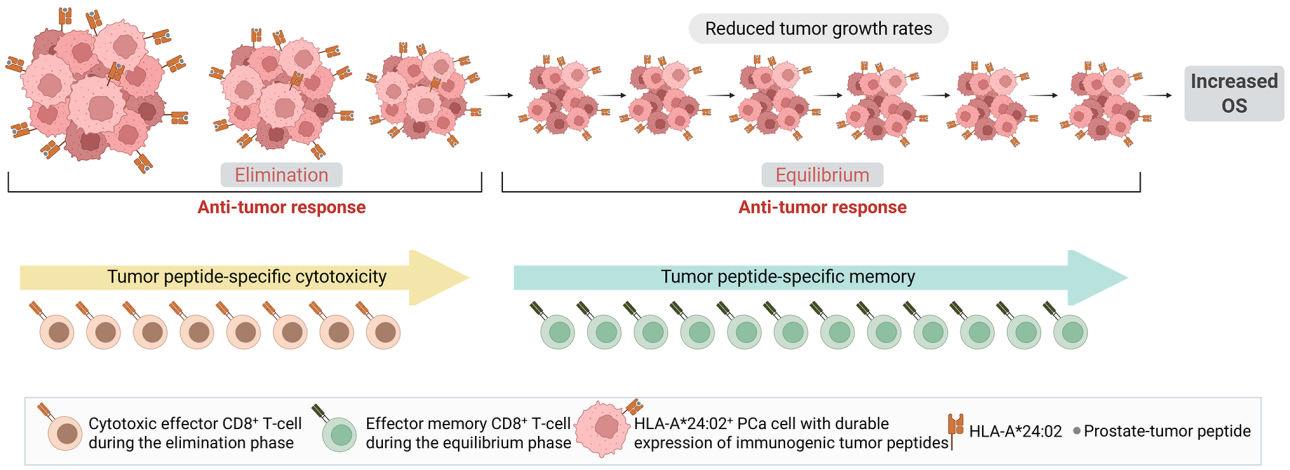

Figure 4. HLA-A*24:02-mediated immunogenicity and durable immune control in PCa. Schematic representation of the proposed mechanism by which HLA-A*24:02 expression confers favorable clinical outcomes in PCa. HLA-A*24:02-positive tumor cells maintain sustained presentation of tumor-derived peptides throughout disease evolution, resulting in enhanced immunogenicity and persistent activation of tumor-specific CD8+ T-cell memory. This continuous immune recognition supports long-term immunosurveillance and establishes an extended state of immune 'equilibrium,' characterized by reduced tumor growth rates and improved OS [Created in BioRender. Fortis, S. (2026) https://BioRender.com/3dl9c85]. Agreement license VD29LKOJ3P. HLA, human leukocyte antigen; PCa, prostate cancer; OS, overall survival.

Tregs and MDSCs) and inhibitory cytokines are ineffective even if present (206,209-211).

Antigen presentation and tumor peptide recognition reflect two other contributing factors for a potent preI, given that effective antitumor immunity requires tumor antigens to be presented to T cells. Tumors that already generate neoantigen-specific T cell signals so that their antigen-presentation machinery and draining lymph nodes are engaged are a favorable setting for therapies that boost T-cell activity. Tumors that evade antigen presentation are harder to treat with immunotherapies (186,212). Finally, the 'immune contexture' denotes one more influencing factor for the magnitude of preI. The immune contexture, which reflects the composition, density and spatial organization of immune cells plus cytokine/chemokine patterns in the TME, predicts both the natural history of the disease as well as treatment outcome. A TME dominated by effector T cells and pro-inflammatory signals supports therapy response; by contrast, a TME dominated by suppressive cell types or a stroma that physically excludes immune cells resists therapy (206,213,214).

Consequently, enumerating tumor-infiltrating lymphocytes density, CD8+ T-cell localization, IFN-γ gene signatures and PD-L1/T-cell-inflamed transcriptional profiles are used to predict patients who will likely benefit from immunotherapies (215). For 'cold' tumors (that is, tumors with no infiltration by immune cells), clinicians and researchers combine ICIs with radiation, oncolytic viruses, targeted drugs, cytokines or vaccines, aiming to induce or recruit T cells and convert the tumor into an immune-responsive state. The goal is to create or boost the antitumor preI that determines downstream success (216). Given that antitumor preI reflects a memory immune response, sustained expression of the targeted tumor antigens throughout disease evolution is essential. Only under these conditions can preI be continuously reactivated, thereby contributing to the control of tumor growth. In a recent study (154), patients with PCa across all stages of the disease, who exhibited high frequencies of HER-2/neu (780-788)-specific CD8+ T cells, demonstrated significantly improved PFS compared with patients harboring lower frequencies, despite comparable clinical characteristics and standard-of-care treatment. These HER-2/neu (780-788)-specific CD8+ T cells likely represent an endogenous preI, as evidenced by their functional recognition of the HER-2/neu (780-788) epitope. Notably, their presence in both localized and metastatic disease suggests that this antigen is maintained throughout PCa progression. Collectively, these findings suggest the potential of the HER-2/neu (780-788) epitope as a candidate for therapeutic vaccine developments and indicate that preI against this epitope may serve as a robust prognostic biomarker in PCa.

Moreover, in our previous studies (217-224), a therapeutic vaccine designed against the 15-mer peptide HER-2/neu (776-790) (AE37 vaccine) showed notable clinical benefits in patients with PCa or breast cancer. In the study by Voutsas *et al* (221) in particular, patients with localized and metastatic PCa with preI to the AE37 vaccine *in vivo*, assessed as a dermal reaction at the injection site as early as 48 h after the first vaccination, showed significantly prolonged PFS compared with patients who did not develop *in vivo* AE37-specific response. In parallel, AE37-specific preI was also detectable *in vitro*, as indicated by increased IFN-γ production by patient-derived T cells following short-term stimulation with AE37 prior to vaccination. Patients with detectable baseline *in vitro* preI also experienced improved PFS relative to those without measurable responses. Beyond AE37-specific immunity, these patients displayed HLA-A2 and HLA-A24-restricted CD8+ T-cell preI against additional tumor-associated epitopes, including HER-2/neu (369-377), PSA (146-154), HER-2/neu (85-94), TERT (540-548) and PSA (153-161). This antigen-specific immunity was further amplified during vaccination, indicating robust epitope spreading, both intramolecular (within HER-2/neu) and intermolecular (across PSA and TERT antigens). We propose that the robust vaccine (AE37)-induced activation of AE37-specific CD8+ T cells,

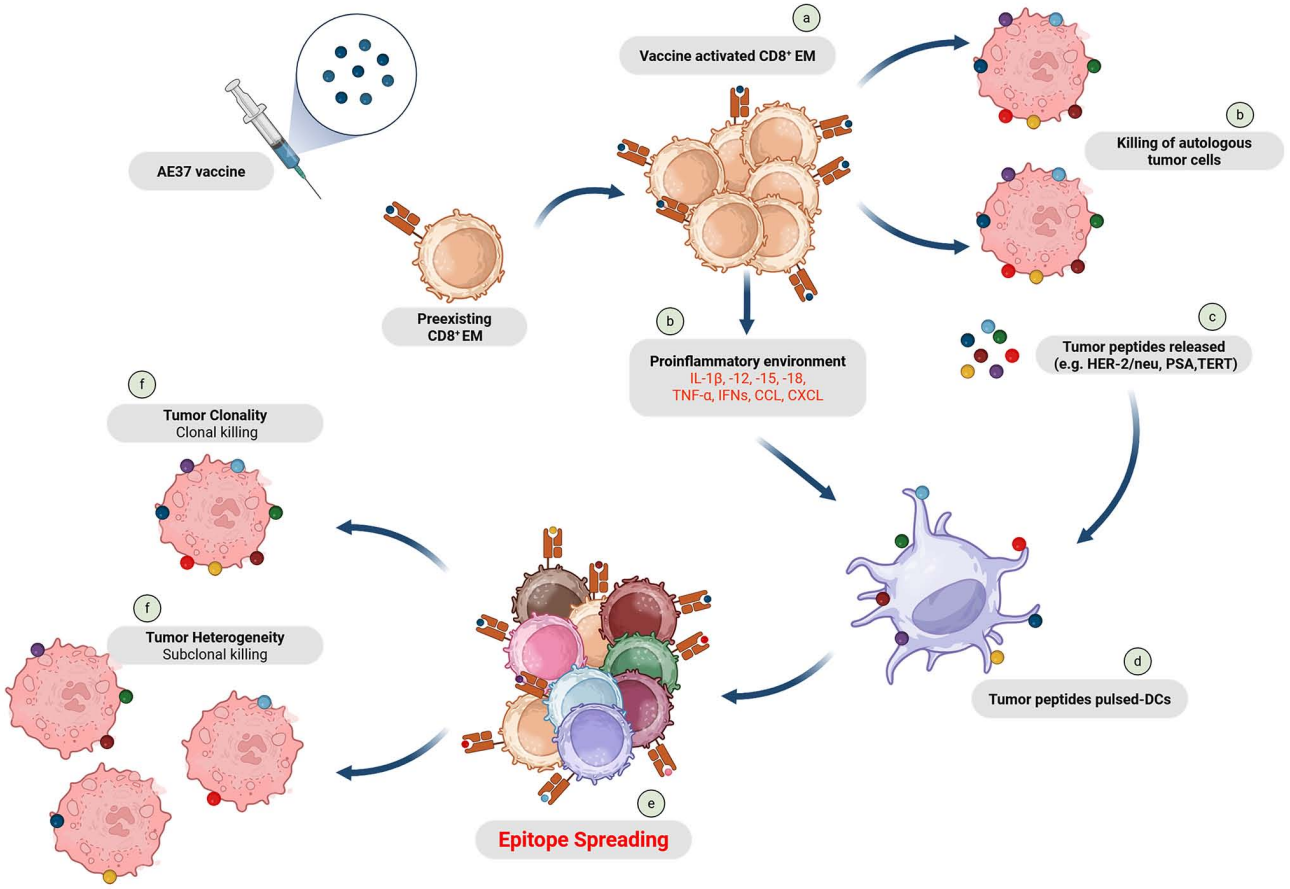

Figure 5. Proposed mechanism of AE37 vaccine-induced antitumor immunity and epitope spreading. Schematic illustration of the AE37 vaccine-driven immune response. (A) Vaccination induces robust activation of preexisting AE37-specific memory CD8+ T cells, leading to the generation of a proinflammatory tumor microenvironment and (B) direct lysis of autologous tumor cells expressing epitopes encompassing the AE37 amino acid sequence. (C) Tumor cell lysis results in the release of additional tumor-derived peptides, including HER-2/neu, PSA and TERT, which are subsequently (D) captured and cross-presented by DCs to other preexisting tumor-specific memory CD8+ T cells bearing cognate T-cell receptors. (E) This process promotes secondary memory CD8+ T-cell activation, clonal expansion and amplified tumor cell killing across both (F) clonal and subclonal populations, thereby overcoming tumor heterogeneity [Created in BioRender. Fortis, S. (2026) https://BioRender.com/mydl63k]. Agreement license DI29LKOS9N. EM, effector memory; CCL, chemokine (C-C motif) ligand; CXCL, chemokine (C-X-C motif) ligand; PSA, prostate-specific antigen; TERT, telomerase reverse transcriptase; IL, interleukin; DCs, dendritic cells.

which generate a proinflammatory milieux and additionally lyse autologous tumor cells expressing epitopes encompassing the amino acid sequence of AE37. Furthermore, lysed tumor cells release their tumor peptides, including HER-2/neu, PSA and TERT peptides, which are cross-presented by dendritic cells to other CD8+ T cells of the preI response carrying specific TCRs, resulting in their activation and expansion and to increased tumor cell killing either clonal or subclonal due to tumor heterogeneity (Fig. 5).

PSA (153-161) appears to have a broad role as a tumor peptide target for preI in PCa. In our more recent report (225), prognostic biosignatures encompassing peripheral blood transforming growth factor (TGF)β, interleukin (IL)-8 and HER-extracellular domain (ECD) levels combined with PSA (153-161)-specific CD8+ T-cell frequencies in patients with localized PCa (LPCa) could be identified. Low levels of TGFβ, IL-8 and HER-ECD comprised a favorable signature that was associated with increased PSA (153-161)-specific CD8+ T-cell frequencies and increased patient survival. By contrast, patients with LPCa and high levels of TGFβ, IL-8 and HER-ECD, had decreased PSA (153-161)-specific CD8+ T-cell frequencies and unfavorable survival rates (Fig. 6). Given that TGFβ, IL-8 and HER-ECD have been demonstrated to exert suppressor functions in PCa and other types of cancer (226-229), their presence in the periphery at low levels likely allows the development of a more potent antitumor immunity, resulting in favorable clinical outcomes. However, their presence at high levels in blood circulation can be linked to tumor progression, metastasis and immune evasion. It is also plausible that the levels of suppression in the periphery regulate the densities of preexisting PSA (153-161)-specific CD8+ T-cells and through them, they significantly influence the clinical course of patients.

Across multiple studies, evidence shows that preexisting tumor-peptide-specific CD8+ T cells represent a powerful prognostic biomarker in PCa as they reflect a primed, antigen-experienced immune system capable of rapid reactivation by therapies such as vaccines, ICIs, radiation or targeted agents. The quality, density, localization and clonality of these T cells, together with a competent antigen presentation process and a supportive immune contexture, determine whether a tumor is 'inflamed' and thus responsive to immunotherapy. Findings from the HER-2/neu (780-788) and AE37 vaccine studies demonstrate that patients with higher baseline

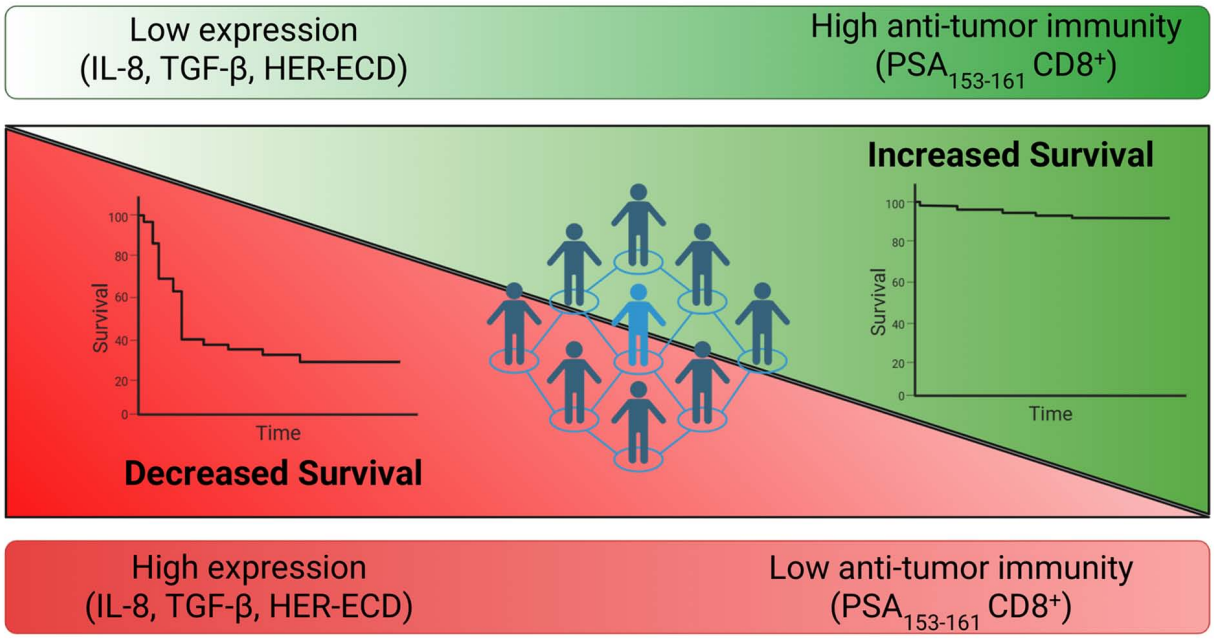

Figure 6. Peripheral blood prognostic biosignatures integrating soluble factors and tumor-specific CD8+ T cells in localized PCa. Schematic representation of prognostic immune biosignatures in patients with localized PCa based on circulating levels of TGFβ, IL-8 and HER-ECD in combination with PSA (153-161)-specific CD8+ T-cell frequencies. Low peripheral levels of TGFβ, IL-8 and HER-ECD define a favorable biosignature associated with increased frequencies of PSA (153-161)-specific CD8+ T cells, enhanced antitumor immunity and improved patient survival. By contrast, elevated circulating levels of TGFβ, IL-8 and HER-ECD characterize an unfavorable biosignature marked by reduced tumor-specific CD8+ T-cell frequencies, immune suppression, tumor progression and poorer clinical outcomes. These findings suggest that the degree of peripheral immune suppression regulates the abundance of preexisting tumor-specific CD8+ T cells and thereby significantly influences disease course and prognosis [Created in BioRender. Fortis, S. (2026) https://BioRender.com/unh610m]. Agreement license ZF29LKOZWI. PCa, prostate cancer; TGFβ, transforming growth factor-β; IL-8, interleukin-8; HER-ECD, HER extracellular domain; PSA, prostate-specific antigen.

frequencies of tumor-specific CD8+ T cells consistently experience a longer PFS time, show broader epitope spreading and mount stronger vaccine-induced responses, while suppressive factors such as high TGFβ, IL-8 and HER-ECD correlate with reduced tumor-specific T-cell frequencies and poorer outcomes. Collectively, these data support the concept that measuring preexisting tumor peptide-specific CD8+ T cells offers a robust, biologically grounded predictor of prognosis and therapeutic responsiveness in PCa and highlight their potential utility in guiding personalized immunotherapeutic strategies.

8. Conclusions and perspectives

PCa management is rapidly moving away from one-size-fits-all therapeutic algorithms toward a layered, precision approach that integrates clinical variables, imaging and multimodal biomarkers. Established tools (including mpMRI, PHI/4K and genomic-based prognostic assays such as OncotypeDX, Prolaris and Decipher) already improve risk stratification and reduce overtreatment in localized disease, while liquid-biopsy modalities (such as monitoring CTCs, cfDNA/ctDNA and EVs) and tissue immune signatures deepen real-time understanding of tumor biology in advanced settings and germline HLA genotype provides an upstream, mechanistic layer that links these domains. It remains clear that no single biomarker class is sufficient to fully capture the complexity of tumor-immune interactions in PCa. However, most promising signals described in the present review remain at different stages of maturity; some (ctDNA and CTC enumeration/profiling) have clinically actionable roles in selected metastatic disease contexts (such as molecular profiling or prognostication), although their use remains complementary to standard diagnostics rather than universally guideline-mandated, whereas others (EV-metabolomics, exosomal miRNA and proteomic panels, HLA-allele prognostication and immune spatial signatures) need standardization, larger validation cohorts and prospective outcome data before their clinical routine adoption.

To translate these advances into improved patient outcomes we recommend three parallel priorities. First, rigorous technical and pre-analytical harmonization. Standardized sample collection, validated analytical pipelines (UMIs with error suppression for ultra-deep ctDNA, EpCAM-independent CTC capture and unified EV isolation/workflows) and cross-platform calibration are essential to reduce variability that currently limits clinical utility. Second, prospective, multimodal cohort studies and biomarker-driven trials that combine germline HLA-typing, tumor genomics /transcriptomics, immunopeptidomics, spatial immune profiling, circulating biomarkers and detailed clinical follow-up. Only longitudinal, integrative datasets can prove causality (for example, HLA-driven immunoediting and allele-specific LOHHLA), identify who benefits from checkpoint blockade or vaccines and justify changes in a patient's care. Third, rational therapeutic development guided by biomarkers, including allele-aware vaccine and neoantigen strategies, combination regimens to convert 'cold' tumors to 'hot' (including radiation,

oncolytics, epigenetic or cytokine priming plus ICIs) and approaches that bypass MHC-dependence when tumors acquire antigen-presentation defects (such as NK cell-based therapies, bispecifics and CAR approaches). In particular, the HLA-A24 vs. HLA-A2 signals warrant expanded, ancestry-diverse validation and mechanistic work (including immunopeptidomics and functional T-cell assays) to determine whether these associations are causal and whether HLA genotypes could ultimately inform patient selection or vaccine design.

Several practical caveats must accompany optimism. Biomarker performance is disease-stage dependent (ctDNA and some CTC measures are far more informative in mCRPC than in early localized disease), population stratification and ancestry effects can confound germline associations, predictive algorithms based on *in silico* binding need orthogonal functional confirmation and cost, accessibility and equity will determine the real-world impact of any new test. Therefore, health-economic analyses, inclusion of underrepresented populations and early engagement with regulatory and reimbursement stakeholders should parallel scientific validation.

In summary, the future will likely see precision PCa care delivered by integrated pipelines that combine refined imaging, validated tissue immune signatures, sensitive liquid-biopsy readouts and germline/tumor HLA/neoantigen information to personalize screening, surveillance and therapy. Achieving this vision requires coordinated efforts, technical standardization, large, multimodal longitudinal cohorts, prospective biomarker-stratified trials and mechanisms to ensure equitable access, but the payoff is substantial including fewer unnecessary interventions, earlier detection of aggressive tumor biology and smarter, immune-aware therapies that prolong meaningful patient survival with less toxicity. Future biomarker frameworks should therefore move beyond parallel evaluation toward true integration, where germline HLA variation, tissue immune architecture and liquid biopsy dynamics are jointly modeled to capture the full continuum of tumor-immune co-evolution in PCa. Translating liquid biopsy technologies into routine care will require not only technical refinement but also demonstration of clear clinical benefit, cost-effectiveness and integration into evidence-based guidelines. Notably, future progress will depend not only on identifying favorable biomarkers but also on rigorously addressing conflicting evidence, understanding mechanisms of immune resistance and determining how these factors limit the clinical efficacy of immunotherapies in PCa.

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CNB, OET and ADG conducted the literature search and drafted the manuscript. CNB, SS, SPF and MG and edited the figures. CNB, SS, SPF, MG, OET and ADG critically reviewed and edited the manuscript. All authors read and approved the final version of the manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

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Competing interests

The authors declare that they have no competing interests.

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